Ryuso TANAKA* & Kazuo OGINUMA*: **Karyomorphological studies on *Clethra barbinervis* and two allied species**

田中隆荘*・荻沼一男*: リョウブおよび2近縁種における核形態学的研究

It is one of the subject in the study of plant evolution that how a species is fixed in the static state of phylogenetic development without showing variational and speciating divergence. The present *Clethra barbinervis* seems to be one of such species fixed in a stable state in phylogenetic development, since in the genus *Clethra* only one species, *Clethra barbinervis* Sieb. et Zucc., is known natively in Japan, while observations on this plant have been made frequently by plant taxonomists.

According to the observations in Orchidaceae, the morphological features of chromosomes at resting phase clearly differ between the species which show rapid speciation and those of slow speciation (Tanaka 1971). From these facts, the morphology of chromosomes in whole phases of cells was considered to have close correspondence to the evolutional property of plant species (Tanaka 1971, 1978). The present investigation was carried out as one of the studies of the evolutional significance of chromosome morphology.

Materials and methods *Clethra barbinervis* Sieb. et Zucc. was collected from four localities in Hiroshima Pref. of Japan. In comparison with the Japanese species two species from North America, *C. acuminata* Michx. and *C. alnifolia* L., were investigated karyomorphologically, and they were listed in the Table 1. The two species from North America were obtained from the Cary Arboretum of the New York Botanical Garden. They were germinated in the Miyajima Botanical Garden attached to the Faculty of Science, Hiroshima University. We thank to Dr. T. Seki of the Miyajima Botanical Garden for his help in collecting two North American species.

For the observation of chromosomes, young leaves of about 2 mm length were pretreated with aqueous 0.002 M 8-hydroxyquinoline at room temperature (about 20°C) for about four hours and fixed in modified Carnoy's solution (99% ethanol : chloroform : glacial acetic acid=2:1:1) for over one hour at

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about 10°C. The fixed leaves were then stained and macerated at the same time with a mixture of 10 parts of 2% aceto-orcein+1 part of 1N HCl applying the squash method.

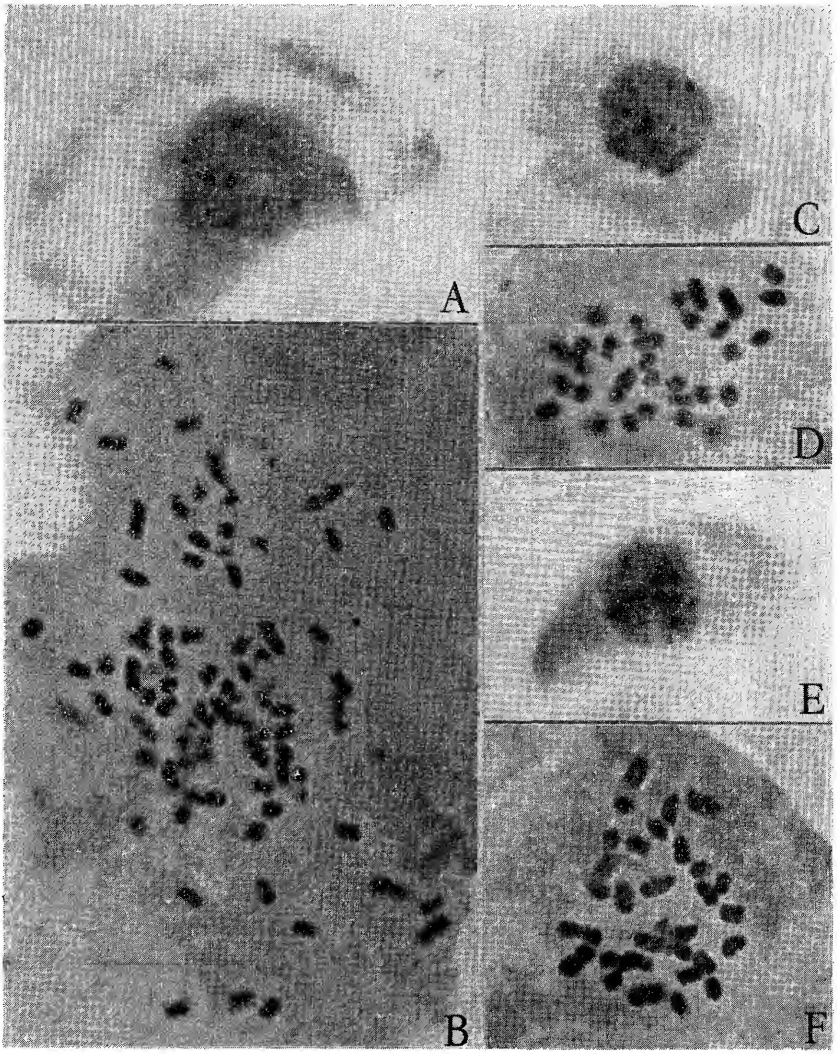

Fig. 1. Photomicrographs of somatic chromosomes in three species of *Clethra* at resting stage (A, C, E) and at mitotic metaphase (B, D, F). A and B: *C. barbinervis* (2n=80). C and D: *C. acuminata* (2n=32). E and F: *C. alnifolia* (2n=32). ×2400.

Validated specimens of the chromosome observations were deposited in the Herbarium of the Botanical Institute of Hiroshima University.

Observations Many mitotic cell divisions were observed in the young leaves. Observations on the morphology of chromosomes were done in both resting and mitotic chromosomes in the parenchymatous and meristematic cells of young leaves.

1. *Clethra barbinervis* Sieb. et Zucc.

Chromosomes in the resting nuclei formed chromatin threads and many chromomeric granules scattered in nuclear space. Several chromocentral small blocks were formed in the resting nuclei. They varied in number from 8 to 10 per nucleus and were mostly round in shape with about 0.3-0.6 μm in diameter. The chromocentral blocks uniformly distributed within the nucleus (Fig. 1-A). The morphology of chromosomes at resting phase was found to come under the category of the simple chromocenter type proposed by Tanaka (1971).

At metaphase $2n=80$ chromosomes were counted in all of the four plants from four localities (Table 1). These chromosomes varied in length from about 0.7 μm to 2.2 μm. Ten of them, being about 2.2 μm, were clearly longer than other chromosomes. The shorter 70 chromosomes ranged gradually from about 0.7 μm to 1.7 μm. The chromosomes showing the heterogeneous variation are categorized as the bimodal karyotype in chromosome length. The longer ten chromosomes had the centromere situated in the median position. Two or three of them had the small constriction in the long arm. Shorter 70 chromosomes had the centromere situated in the median or submedian position. The chromosome complement was categorized to be the symmetric karyotype in arm-ratio. Satellites were found in six chromosomes which were medium in length (Figs. 1-B and 2-A). The satellites were observed in the short arms forming a subterminal secondary constriction, respectively.

2. *C. acuminata* Michx.

Chromosomes in the resting nuclei formed chromatin threads and many chromomeric granules scattered in nuclear space. Several chromocentral round blocks were formed in the resting nuclei. They varied in number from 8 to 10 per nucleus and were about 0.3 μm-0.8 μm in diameter (Fig. 1-C). Thus, the morphology of chromosomes at resting phase was categorized to be the simple chromocenter type which was similar to that of the previous

species, *C. barbinervis*.

At metaphase 2n=32 chromosomes were counted. They varied in length from about 0.7 μm to 1.8 μm. Four of them, being about 1.8 μm, were clearly longer than other chromosomes. The remaining 28 chromosomes ranged gradually from about 0.7 μm to 1.0 μm. The chromosomes of a complement showing the heterogeneous variation are categorized as the bimodal karyotype in chromosome length. The longer four chromosomes had the centromere situated in the median position. One or two chromosomes of them had the small constriction in the long arm. Shorter 28 chromosomes had the centromere situated in the median or submedian position. Satellites were found in the two chromosomes which were medium in length (Figs. 1-D and 2-B). These satellites were observed in the short arms forming a subterminal secondary constriction in both of the chromosomes.

3. *C. alnifolia* L.

Morphology of chromosomes in the resting nuclei were similar to those of the previous two species, *C. barbinervis* and *C. acuminata*. Thus, it was categorized to be the simple chromocenter type (Fig. 1-E).

At metaphase 2n=32 chromosomes were counted. It confirmed the report of Hagerup (1928). 2n=32 chromosomes had the four longer chromosomes and 28 shorter chromosomes which ranged gradually. The longer four

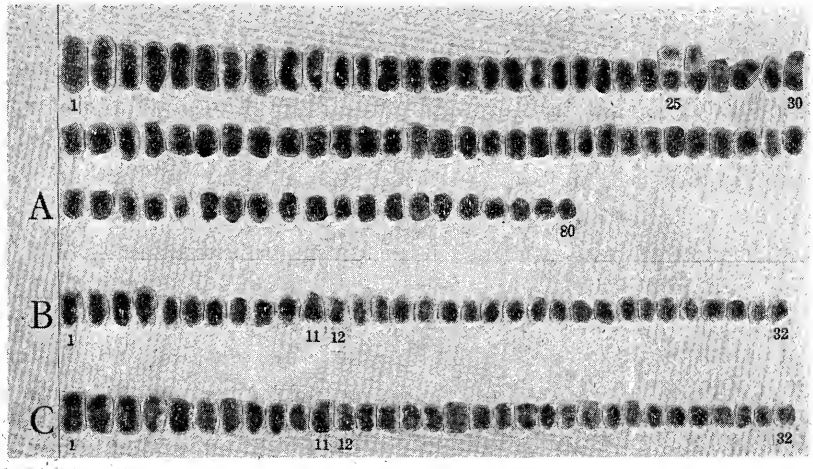

Fig. 2. Photomicrographs of chromosomes at mitotic metaphase in three species of *Clethra*. A: *C. barbinervis* (2n=80). B: *C. acuminata* (2n=32). C: *C. alnifolia* (2n=32). ×2700.

chromosomes had the centromere situated in the median position. The 28 chromosomes had the centromere situated in the median or submedian position. Satellites were found in the two chromosomes which were medium in length. The satellites were observed in the short arms forming a subterminal secondary constriction in both of the chromosomes (Figs. 1-F and 2-C). The chromosomes at metaphase were found to be similar in karyotype to those of previous *C. acuminata*.

Table 1. Localities and chromosome numbers of *Clethra* investigated

Species	Locality	Chromosome number (2n)
C. barbinervis Sieb. et Zucc.	Ōno T., Hiroshima Pref.	80
	Miyajima Is., Hiroshima Pref.	80
	Higashihiroshima C., Hiroshima Pref.	80
	Sandan-kyo, Hiroshima Pref.	80
C. acuminata Michx.	Transylvania County, North Carolina	32
C. alnifolia L.	Millbrook, Dutchess County, New York	32

Discussion *C. barbinervis* occurs commonly in Japan distributed widely from Hokkaido to Kyushu. In Japan only this species has been reported as wild (Makino and Nemoto 1931, Nemoto 1936, Hara 1948, Kitamura and Murata 1971, Ohwi 1978). In the view of plant speciation this species can be regarded as a stable species fixed in the static state of speciation.

The chromosome number 2n=80 of *C. barbinervis* has been observed in the present investigation. The same chromosome number was found in all of the four localities of coastal, inland and mountainous regions, in Hiroshima Prefecture. Therefore, *C. barbinervis* is considered to be 2n=80 at least in western Chugoku District.

In *Clethra* about 30 species have been reported in the world. Only three of them have been studied cytologically and their chromosome numbers are reported to be *C. arborea* 2n=16 (Hagerup 1928), *C. lanata* n=8 (Kyhos 1965) and *C. alnifolia* 2n=32 (Hagerup 1928, present investigation). In the present investigation 2n=80 of *C. barbinervis* and 2n=32 of *C. acuminata* were newly counted. Among these chromosome numbers a polyploid series with the basic number x=8 is found as follows: the diploid with 2n=16, the tetraploid with 2n=32 and the decaploid with 2n=80. Therefore, in the genus *Clethra* an

interspecific polyploidy has occurred widely in the world and *C. barbinervis* in Japan has been found to be the highest, decaploid, of them.

The chromosome complements of metaphase chromosomes in the tetraploid and decaploid species showed the morphological features as follows. In both tetraploid species, *C. acuminata* and *C. alnifolia*, four longest chromosomes which differ conspicuously from the other 28 chromosomes were observed. These four chromosomes showed similar shape in the position of median centromere. The basic number x=8 of the two tetraploids seems to be derived from the complement having one long chromosome. In decaploid *C. barbinervis*, ten longest chromosomes which were similar to those of the tetraploid species were observed. *C. barbinervis*, therefore, seems to have derived from the same basic number x=8 with the tetraploid species. The secondary constrictions and small constrictions were observed in shorter chromosomes in the three species. The shape of metaphase chromosomes also resembled each other in respect to heterogeneous variation of size and symmetric arm-ratio. In addition to this the resting chromosomes of the three species were observed to be the same simple chromocenter type with several round chromocenters, and the number of chromocenters in a nucleus was found to be the same between the tetraploid and decaploid. According to these karyomorphological features *C. barbinervis* is considered to be the decaploid species which was not markedly differentiated from the two tetraploid North American species, *C. acuminata* and *C. alnifolia*. These karyomorphological features, particularly the decaploidy, might have played an important role for the fixation of *C. barbinervis* in the static state of speciation.

The genus *Clethra* is a small genus speciating into about 30 species in the world. For such poor speciation as in *Clethra* the karyomorphological features of the species of this genus were found to be heterogeneous in chromosome size, symmetric in arm-ratio and simple chromocenter type at resting phase. The problems of the interrelationship between the karyomorphological features and the speciating characteristics will be treated in the following papers.

Summary

1. Karyomorphological studies were carried out in *Clethra barbinervis* Sieb. et Zucc. from Japan, *C. acuminata* Michx. and *C. alnifolia* L. from

North America.

2. *C. barbinervis* was found to be 2n=80, and *C. acuminata* and *C. alnifolia* were 2n=32.

3. The three species were found to have similar karyomorphological features showing a bimodal and heterogeneous type in chromosome length, symmetric type in arm-ratio, satellited shorter chromosomes, and simple chromocenter type in the morphology of resting chromosomes.

4. The 2n=80 of *C. barbinervis* had ten markedly longer chromosomes, and both the 2n=32 of *C. acuminata* and the 2n=32 of *C. alnifolia* had the four markedly longer chromosomes.

5. *C. barbinervis* was considered to be decaploid, and *C. acuminata* and *C. alnifolia* tetraploid with the same basic number x=8.

Literature cited

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Clethra barbinervis リョウブは本邦では1属1種であり，種分化が停滞している種の一つである。本種と北米産 *C. acuminata* と *C. alnifolia* との核形態学的研究を行い，次の結果を得た。染色体数は，*C. barbinervis* が 2n=80, *C. acuminata* と *C. alnifolia* がともに 2n=32 であった。3種は，ともに，静止期染色体が単純染色中央粒型，分裂期染色体が二様相的非均等染色体長および対称的腕比，同形の付随体染色体をもっていた。また，*C. barbinervis* は，2n=80 中に長い染色体を10個，*C. acuminata* と *C. alnifolia* は，ともに 2n=32 中に *C. barbinervis* と同形の長い染色体を4個もっていた。核型，および 2n=16 の他種 (*C. arborea*, *C. lanata*) の存

在から，*C. barbinervis* は x=8 の10倍体であり，*C. acuminata* と *C. alnifolia* とは x=8 の 4 倍体であるとみなされる。*C. barbinervis* における種分化の停滞は，このような核形態学的特質と関係があると思われる。

□西澤一俊・千原光雄(編)：**藻類研究法**　754 pp. 1979. 共立出版. ¥12,000. この書は編集者を含めて 29 名の若者によって執筆されたものである。内容は序論—藻類研究の歴史的背景と将来の展望，次いで本論として藻類の分離と培養，藻類の形態と細胞の観察法，藻類生理生態研究法，藻類の生化学的研究法の 4 章から成り，付録として藻類の分類，分類表，種の同定の参考書，それに和名・学名・培地名・事項の別に詳細な索引がついている。

各章では基本的事項・操作の解説につづいて各論に入っているが，各論のところは扱われいる実験例も多く，しかも一つ一つが詳細である。たとえば，藻類の分離と培養では，培養研究の基本的事項につづいて，アオサなど 11種の大型藻と，ボルボックス他 4 種の微細藻の培養例が，そして藻類の形態と細胞の観察の項では，基本的な器具やその操作法，各論としては，原形質連絡糸，鞭毛，細胞壁，核と染色体などの具体的な観察方法が述べられ，走査型電顕による観察にまで及んでいる。生理生態研究法でも現存量，光合成，呼吸などのいろいろの測定法の解説にとどめず，大型藻・微細藻の測定例が詳細に述べられている。また，環境要因の測定では，最近注目されている水界汚染と関連して，藻類を利用した水質評価の問題にもふれている。生化学的研究法では光合成色素，酵素，同化物質，細胞壁構成物質，二次代謝物質のそれぞれについて，多くの例を挙げて抽出・検定・同定を述べ，藻類を対象にしたものとしては新しい分野である植物ホルモン，抗菌性物質，受精物質と性ホルモンにまで及んでいる。

従来，研究法，実験法と銘打った書物は数多く出ているが，対象を大型藻を含む藻類にしぼって，これほどまでに関連分野を網羅し，しかも多くの具体例を挙げて，その実験法，研究法を述べたものは，これが最初であろう。しかも，著者がそれぞれの分野で現在活潑に研究活動をしている人達であるだけに，単なる研究法解説ではなくて，著者達が工夫をしながら研究を進め，体得された'こつ'が極めて合理的な形で述べられている。入門を志す人には勿論，専門家にとっても誠に有益であり，編者の意図された“藻類研究法の手引になるような基本書”として，常時利用される書であろう。

さらに，この書の大きな特色は，分野・事項ごとに目的，意義と問題点などの 1 章を設け，それぞれの実験・研究の目的や，その結果が藻類学研究の中で，どの様に位置し，どの様な意味をもつかが，一つ一つ明快に述べられている点である。いうまでもなく，実験・研究はデーターを出すこと自体が目的ではなく，そのデーターの解析，意義づけが目的であるが，そうした点にまで及んでいることは，本書の価値をさらに高めるものであろう。　　(山岸高旺)